

Small Pox Specimen Information for Utah Department of Health Laboratory
Please contact Dr. Pounder (801) 584-8449 prior to submitting the specimen for testing.

The Utah Department of Health Laboratory can test for the presence of Variola virus, Vaccinia virus, and Varicella Zoster virus at this time. Outlined below are guidelines for specimen collection and transport. Please handle all potential specimens with care.

Specimen Handling

A. Specimen Collection-Safety

1. Only personnel who have been successfully vaccinated against smallpox in the last 3 years or non-vaccinated personnel with no contraindication to vaccination should participate in specimen collection. Vaccination of non-vaccinated personnel should occur as soon as possible after specimen acquisition.

2. All procedures for obtaining, processing, packing, and shipping potentially infectious material should be performed by using BSL-2, or if available, BSL-3 practices. Appropriate respiratory and barrier protective equipment such as N-95 or HEPA-filtered mask, gloves, gown, shoe covers, and protective eyewear should be used in specimen collection for suspected cases of smallpox.

3. While working with specimens, laboratory personnel should avoid any activity that brings hands or fingers in contact with mucosal surfaces, such as eating, drinking, smoking, adjusting contacts, or applying makeup.

4. After removing gloves, personnel should thoroughly wash their hands with antimicrobial soaps before leaving the laboratory. Areas of skin known or suspected to have come in contact with variola virus should be washed with the recommended soap, followed by 60-95% ethanol containing gel (available from Fisher Scientific (800) 766-7000). If possible, skin should be decontaminated with 0.5% sodium hypochlorite for at least one minute.

B. Specimen Collection

Collection Kit Guidelines

Test Request form available at www.health.utah.gov/els/microbiology in the Microbiology Client Services Manual.

Personal Protective Equipment: gloves, gown, shoe covers, N95 mask, and eye protection

Alcohol wipes

Scalpel with a number 10 blade

26 gauge needle

Wooden applicator

1.5 to 2 ml screw capped plastic sample tubes

snap capped plastic sample tubes

formalin for fixed tissues (Sigma (800) 325-3010))

clean glass microscope slides

50 ml plastic conical centrifuge tubes for holding glass slides or commercial slide holders

Polyester swabs

Permanent marking pens for labeling tubes

Tiger-top vacutainer tube for serum collection 10cc

Purple-top vacutainer tube for whole blood collection 5cc

Vacutainer collection supplies

Sharps containers

M4 transport medium (REMEL (800)255-6730)

Gauze

Most items are available from Fisher Scientific (800)766-7000, VWR (800)932-5000 or ISC BioExpress (800)999-2901

Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources; inclusion does not imply endorsement by the Utah Department of Health.

1. Label all tubes, vials, and microscope slide holders with patient's name, unique identifier, date of collection, source of specimen (vesicle, pustule, scab, or fluid), and name of person collecting sample.

2. Avoid cross-contamination of samples. Use one sample per primary container. Collect sufficient amount of lesion material to permit multiple diagnostic tests and confirmations. Suitable specimens for virologic tests of suspected smallpox cases are biopsy tissues, 2 to 4 scabs, and vesicular tissues and fluid. Serologic testing requires at least 1 ml of serum. Other potentially useful specimens for viral sampling are throat swabs and whole blood, however, quality-controlled diagnostic tests have not been completed for these specimens.

3. Biopsy specimens of individual lesions should be made with a 3.5 or 4.5 mm punch biopsy device. This should sample the entire lesion. The biopsied material should be bisected with sterile scissors or scalpel and placed into 2 labeled containers. One half of the sample should be placed in formalin for immunohistochemical or histopathological evaluation, this container should be kept at room temperature. The other half of the lesion material should be placed in a dry, sterile 1.5 to 2 ml screw capped sample tube, do not add transport medium. Refrigerate these containers if shipment occurs within 24 hours. If shipment will be longer than 1 day, freeze this sample.

4. Scabs should be removed by using a sterile 26 gauge needle and collected in dry, sterile 1.5 to 2 ml screw capped sample tube.

5. Vesicular material should be sampled after the skin area has been sanitized with an alcohol wipe and allowed to dry. Unroof the lesion with a sterile 26 gauge needle or with a scalpel. Place the skin "roof" in a dry, sterile 1.5 to 2 ml screw capped sample tube. Scrape the base of the blister with a wooden applicator and smear the scrapings onto a clean glass light-microscope slide. Touch a clean glass light-microscope slide to the open lesion multiple times. If additional tests are required electron microscope slides will be provided. Lightly touch the shiny side of 1 or 2 plastic coated grids to the base of the open lesion. Allow slides and grids to air dry for approximately 5 minutes, then place in appropriate containers. Repeat this procedure for 2 or more lesions.

6. Autopsy specimens from major organs collected for virus isolation and immunohistochemical and histopathological evaluation include skin, spleen, lymph node, liver, lung, kidney, and heart. Specimens for virus isolation should be frozen. Specimens for immunohistochemical and histopathological evaluation should be fixed in formalin.

7. Blood should be drawn into a purple-topped tube for possible viral identification and into a marble-topped serum separator tube for serological testing.

8. A cotton or polyester swab should be used for sampling tonsillar tissue in the posterior pharynx. Collect the specimen as a throat swab. Break off the end of applicator into a 1.5 to 2 ml screw capped sample tube. Do not add transport medium.

9. Package each patient's lesion specimens separately to avoid cross-contamination. See the next section for sample transport information.

10. When specimen collection has been completed, all protective materials and sample collection materials must be double bagged in biohazard bags and autoclaved or incinerated.

11. Specimens for Variola virus testing are dried vesicular fluid on a microscope slide, vesicular tissue (skin from unroofed vesicle), swab of vesicular fluid. Specimens for Variola virus PCR should be dried vesicular fluid on slide, scab or swab material. To obtain a microscope slide preparation, unroof the scab with a sterile scalpel or a 26 gauge needle. Place the scab in a labeled plastic sample tube, do not add transport medium. Touch a clean glass microscope slide to the open lesion several times. Allow the slide to air dry and place in an appropriate labeled container. Swabs of vesicular fluid should be collected by vigorously scrubbing the base of an unroofed lesion with a sterile swab. A polyester swab is preferred. Contamination with blood is not a concern for this test. Place swab in a snap cap tube or other suitable container. Break off the stick if necessary. Do not add transport fluid. Specimens for PCR testing, may be stored indefinitely and shipped at room temperature.

12. Specimens from cases that require rule-out testing for Varicella Zoster Virus (VZV) will be analyzed by the direct fluorescent antibody (DFA) method. To collect specimens for the DFA test, unroof the vesicle using a 26 gauge needle. Scrub the base of the lesion vigorously enough with a sterile swab to collect lesion cells. Avoid contaminating the sample with blood. Place the swab in a sterile tube containing 1 to 2 ml of suitable transport medium (M4 viral transport medium) Keep swab moistened. Transport with a cold pack, do not freeze. Specimens for VZV PCR should be scab or swab material. Collect by lifting the scab using the beveled point of a sterile 26 gauge needle and place in a snap cap tube or other suitable container. Do not add transport medium. Swabs of vesicular fluid should be collected by vigorously scrubbing the base of an unroofed lesion with a sterile swab. A polyester swab is preferred. Contamination with blood is not a concern for this test. Place swab in a snap cap tube or other suitable container. Break off the stick if necessary. Do not add transport fluid. Specimens for PCR testing, may be stored indefinitely and shipped at room temperature.

13. Specimens that require testing to rule-out Vaccinia virus will be analyzed by PCR. To obtain a microscope slide preparation, unroof the scab with a sterile scalpel or a 26 gauge needle. Place the scab in a labeled plastic sample tube. Touch a clean glass microscope slide to the open lesion several times. Allow the slide to air dry and place in an appropriate labeled container.

C. Specimen Handling-Shipping

1. Specimens should be packed using the "triple" packaging scheme of primary receptacle, water-tight secondary packaging and durable outer packaging. Adequate absorbent material will be packed with the specimens to contain all fluids. A rigid, crush-proof overpack should be used. No transport medium or glycerol will be added to the specimens. Formalin-fixed, electron microscopy grids, and PCR specimens should be stored and shipped at room temperature, not frozen. These specimens should not be packed with dry ice as dry ice vapors may cause a change in the pH of the specimens. Additional specimens should be stored at 2-8°C or frozen according to specific directions for the type of specimen. All IATA regulations should be adhered to for preparing packages, labeling packages, and paperwork to accompany the specimens. Transport by courier should follow established safety guidelines.

***SMALLPOX Pages from the
Microbiology Client Services Manual***

State of Utah Public Health Laboratory

46 North Medical Drive

Salt Lake City, UT 84113-1105

Phone: 801-584-8400 FAX: 801-584-8486

Utah Department of Health

MICROBIOLOGY CLIENT SERVICES MANUAL

State of Utah Public Health Laboratory

GENERAL INSTRUCTIONS

CONTACT US:

ADDRESS, PHONE, FAX, and WEBSITE

State of Utah Public Health Laboratory
46 North Medical Drive
Salt Lake City, UT 84113-1105
Phone: 801-584-8400
FAX: 801-584-8486
Webpage: [HTTP://health.utah.gov/els/microbiology](http://health.utah.gov/els/microbiology)

KEY PERSONNEL

Billing

Bob Anderson

Environmental (Water) Microbiology

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Microbiology Bureau

Barbara Jepson, MPA, MT(ASCP) -- Bureau Director

Dan Andrews, MS, MT(ASCP) -- Section Chief of Bacteriology,

Food Bacteriology, Mycobacteriology, Parasitology

Jana Coombs, BS, M/SV (ASCP) -- Section Chief of Newborn Screening

June Pounder, Ph.D. -- Section Chief of Molecular Biology, and
Bioterrorism coordinator

Tom Sharpton, MS, SM(ASCP) -- Section Chief of Immunology, Virology

Technical Services

Chris Peper, MT(ASCP) -- Section Chief

REPORTING:

You must supply your correct Customer ID Code to receive test results.

Some mail services and couriers are taking a week or more to get your samples to us.

If you are having problems with turn around time for results, check your delivery method.

See individual test for specific reporting criteria and methods.

REQUISITIONS:

Blank request forms with your customer ID code are available from Technical Services
(also see Appendix B for blank forms WITHOUT the customer ID).

All information must be provided. Incomplete requisitions cannot be processed.

SPECIMEN LABELING: See individual requirements under specific test.

*****NOTE: Specimen containers from the State of Utah Public Health Lab have an outdate printed on the label. Do not collect any sample in an outdated container.**

Call Technical Services at 801-584-8204 for a new container.

We do not supply blood collection tubes.

MICROBIOLOGY CLIENT SERVICES MANUAL
State of Utah Public Health Laboratory

LAB TEST – Bioterrorism

TEST	Variola virus (Smallpox)
METHOD	N/A
AVAILABLE	All Clients – Contact UDOH Epidemiology prior to submitting test specimens: (801)538-6191
PATIENT PREP	N/A
SPECIMEN	Microscope slide touch preps, scabs, dried vesicular fluid, vesicular swabs, vesicular tissue
COLLECT IN	Refer to Level A Manual for Agents of Bioterrorism
PROCESSING	Refer to Level A Manual for Agents of Bioterrorism
TRANSPORT	Refer to Level A Manual for Agents of Bioterrorism
TIME CRITICAL	Should be received at our laboratory as soon as possible
LABEL	Patient's full name or unique ID number, and date of collection
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	24 hours
RESULTS	Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	Refer to the Smallpox Specimen Information link on the Microbiology website (health.utah.gov/els/microbiology)
CONTACT	(801) 584-8449: June Pounder or Kim Christensen

BIOTERRORISM TEST REQUEST FORM		FOR LABORATORY USE ONLY LAB#: _____ DATE STAMP: _____	
STATE OF UTAH PUBLIC HEALTH LABORATORY 46 NORTH MEDICAL DRIVE SALT LAKE CITY, UTAH 84113-1105 TELEPHONE: (801) 584-8400 FAX: (801) 584-8486			
TESTING WILL NOT BE PERFORMED UNLESS SLIP IS COMPLETELY FILLED OUT. PLEASE PRINT CLEARLY FOR ACCURACY.			
PATIENT INFORMATION:			
Patient Name (Last, First): _____			
Patient ID #: _____	DATE OF BIRTH (mm/dd/yy) _____ / _____ / _____	AGE: _____	SEX: _____ <div style="display: flex; justify-content: space-around;"> M F </div>
PROVIDER INFORMATION: Provider Code: _____		SPECIMEN COLLECTION DATE (MM/DD/YY) _____ / _____ / _____	
SPECIMEN SOURCE/SITE: <input type="checkbox"/> Blood <input type="checkbox"/> Serum <input type="checkbox"/> Urine <input type="checkbox"/> CSF <input type="checkbox"/> Skin <input type="checkbox"/> Bronchial Wash <input type="checkbox"/> Tissue (specify): _____ <input type="checkbox"/> Fluid (specify): _____ <input type="checkbox"/> Feces <input type="checkbox"/> Wound/Abscess <input type="checkbox"/> Throat <input type="checkbox"/> Swab (specify): _____ <input type="checkbox"/> Sputum <input type="checkbox"/> Lesion <input type="checkbox"/> Scab <input type="checkbox"/> Isolate (source): _____ <input type="checkbox"/> Environmental (specify): _____ <input type="checkbox"/> Food (specify): _____ <input type="checkbox"/> Other (specify): _____		TEST ORDERED: <input type="checkbox"/> <i>Bacillus anthracis</i> <input type="checkbox"/> <i>Burkholderia</i> spp. <input type="checkbox"/> <i>Brucella</i> spp. <input type="checkbox"/> <i>Clostridium botulinum</i> culture & toxin <input type="checkbox"/> <i>Coxiella burnetii</i> <input type="checkbox"/> <i>Francisella tularensis</i> <input type="checkbox"/> Orthopox virus <input type="checkbox"/> Ricin toxin (non-clinical) <input type="checkbox"/> <i>Staphylococcus</i> Enterotoxin B (non-clinical) <input type="checkbox"/> Vaccinia virus <input type="checkbox"/> Varicella zoster virus <input type="checkbox"/> Variola virus <input type="checkbox"/> <i>Yersinia pestis</i> <input type="checkbox"/> Other (specify): _____	
		STATE OF ORIGIN OF PATIENT/SAMPLE _____	
		ADDITIONAL INFORMATION (List pertinent information including presumptive ID)	